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THE DIE-BACK FUNGUS OF PARA RUBBER AND OF CACAO.

(Thyridaria tarda, n. sp.)

IMPERIAL BUREAU OF MYCOLOGY
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BY

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Mycologist, F. M. S.

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HISTORY AND DISTRIBUTION.

The parasitism of the fungus was first investigated by Howard in the West Indies in 1901. In that year this author described a die-back disease of cacao in Grenada and showed that it was caused by a fungus which was identified as *Diplodia cacaoicola,* P. Hennings—a species described by P. Hennings in 1895 on the branches of cacao in the Cameroons. In the light of recent work by Griffon and Maublanc this species is considered to possess several synonyms, the earliest of which dates back to 1892. In tracing the history of the fungus, therefore, it will be necessary to deal with the synonyms in their proper chronological order.

In 1892 Patouillard described from Ecuador a fungus on the pods of the cacao plant which it was said to incrust with a black mass; this fungus was named *Botryodiplodia Theobromae,* n. sp. Prilleux and Delacroix figured and described in 1894 on the roots of cacao in equatorial America a species which was said to be particularly injurious to the plant and which they named *Macrophoma vestita,* n. sp. Then followed Hennings' description of *Diplodia cacaoicola,* n. sp. from the Cameroons; it is under this name that the fungus has been known to cause the die-back disease of cacao in the West Indies. *Lasiodiplodia nigra,* n. sp. was named by Appel and Laubert on cacao in Samoa in 1906. In the same year Wurth found the fungus in Java. In 1909 Griffon and Maublanc, working from the

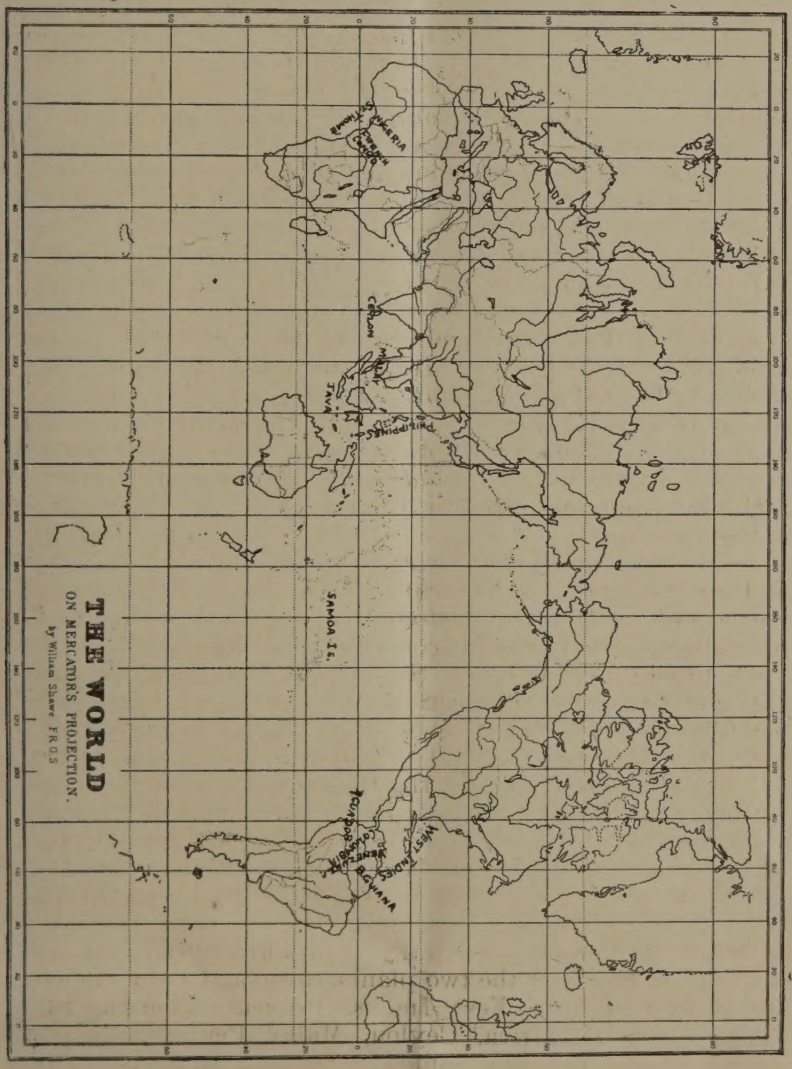
French Congo, showed principally by a comparison of specimens that the above species were all synonymous; these authors also maintained that according to the present system of classification, the fungus was a *Lasiodiplodia* which they named *Lasiodiplodia Theobromae,* preserving the specific name of Patouillard.

More recently other authors have pointed out that other species should be regarded as being synonymous with *Diplodia cacaoicola* or *Lasiodiplodia Theobromae.* Petch, in 1910, described a die-back of Para rubber in Ceylon, the later stages of which he attributed to *Botryodiplodia elasticae,* a species previously named by him on Para rubber in 1906. He regards this species as being in its characters indistinguishable from *Diplodia cacaoicola,* and he points out that a consignment of stumps of *Hevea brasiliensis* which was sent from Ceylon to German West Africa were examined by Brick at Hamburg and were reported to be attacked by *Lasiodiplodia nigra,* which he, therefore, concludes to be the same as *Botryodiplodia elasticae.* South considers that the species described by Stockdale to cause a disease of the stems and roots of cacao in the West Indies in 1909 is identical with *Lasiodiplodia Theobromae* and also with the fungus described by Charles on cacao and mango in San Domingo.

In 1909 a fungus which was reported to cause a disease of Para rubber in the Federated Malay States and Straits Settlements was named *Diplodia rapax,* Massee. By comparing specimens of the fungus which causes the die-back of Para rubber in the Malay States with specimens of the *Diplodia* occurring on cacao in West Africa I concluded that the two were indistinguishable from each other, and, further, as will be described in another part of this work, I have been able to transfer the fungus from cacao to Para rubber and to show that it produces symptoms of disease and fructifications which are identical with those produced by the fungus occurring on Para rubber in this country.

In the above the history of the fungus has been traced from its original description by Patouillard from Ecuador in 1892 to its discovery as a parasite on Para rubber. It occurs almost wherever the two plants, cacao and Para rubber are cultivated,—*i.e.* West Indies, tropical America, St. Thome, tropical Africa, Ceylon, Malay Peninsula, East Indies, Philippines and Samoa. The accompanying map is intended to illustrate the geographical distribution of the fungus, which will be seen to cover an area extending roughly from 20° North to 20° South of the equator, the different countries in which the fungus is known being indicated by their names.

With reference to the distribution of the fungus in the Malay Peninsula, from observations made in the field and from specimens which have been forwarded for examin-



ation to the Department of Agriculture at Kuala Lumpur, I have concluded that it is widely distributed through the Federated Malay States of the Peninsula, in the State of Johore and in Malacca.

The fungus occurs on dying or dead parts of many plants. It can frequently be found on plantations on dead branches of *Hevea;* I have also observed it frequently on dead shoots of coffee, coconut, camphor, *Castilloa elastica* and on dead stems and roots of tapioca. It develops readily on cut surfaces of pineapple, potato or other such material exposed to the air in the vicinity of rubber estates.

SYMPTOMS OF THE DISEASE.

The symptoms are most usually those of a "die back," the infected parts dying back towards the base. In younger plants the main shoot is thus affected; in older plants the leader or one of the lateral shoots commences to die back. When the disease is in its initial stages it seldom happens that more than one or two of the shoots are affected simultaneously. The death of several shoots occurring simultaneously is usually attributable to some other cause, such as root disease.

When a branch is attacked the terminal portion first dies; the length of this terminal portion varies according to the distance of the point of infection from the apex of the branch. The branch continues to die back towards its base and the disease may spread to the trunk and then downwards. Before, however, the disease has spread down the trunk for any considerable distance the death of the other branches of the tree is recorded, owing to the stoppage of the transpiration current. As the branch dies back new shoots are put out below the dead part; these may in their turn succumb to the disease. It not infrequently happens, however, that the dying back of the branch is checked and that growth is continued by one of the shoots which has arisen below the dead portion. After a branch has died back to its base the disease does not always spread to the trunk of the tree. This arrest of the spread of the disease is due to some resistance offered by the plant itself, and the power of resistance is closely related to the general condition of health of the plant.

When the disease has spread down the trunk it continues to spread to the roots of the tree. I have recorded instances in which infection has commenced in the roots themselves. These occurred in stumps which had been recently planted out, and it was concluded that infection had occurred at the cut ends of the lateral roots during the period of the exposure of the roots previous to planting out. It is also possible that in older trees the fungus may effect an entrance into the portions of the lateral roots

which occur above ground, but apart from the instances quoted above in the case of the stumps there has been no record of infection occurring through the roots.

The wood shows symptoms of disease best. In early stages the medullary rays are brown. As the growth of the mycelium proceeds, the whole wood assumes a greyish colour and eventually the colour becomes much darker. The discolouration is almost always homogeneous; it may be more evident on one side than on another, but it never occurs in streaks or lines as is the case with some other fungi of a similar nature.* The death of the wood is always more evident than that of the outer tissues, and, in conjunction with the other symptoms, forms an important method of diagnosis.

After the wood is dead the outer tissues may still yield some latex. These, however, soon die; the bark becomes usually of a greyish colour and finally cracks and peels off. The cambial zone between the bark and wood is eventually reduced to a black mass produced by the growth of the dark coloured mycelium of the fungus.

There is usually a good line of demarcation between dead and dying tissue in the wood as the death of the branch is traced downwards.

Bleeding has been put forward as an indication of the disease. I have only found bleeding to occur in a very small number of the cases examined, and only in a very few of these have I been able to trace the bleeding to a rupture of the tissues caused by the fungus. The rubber plant may bleed from several causes, the two commoner of which are the bending of branches by wind and the pecking of the branches by birds. Bleeding cannot be recognised as a symptom of the die-back disease.

Where two or three of the topmost branches are affected simultaneously the effect simulates in appearance what is known as "stag-head." "Stag-head" occurs when trees, though in good foliage generally, lose the power of bearing leaves on their topmost branches. This condition more frequently arises from other causes, notably when the roots gradually penetrate into unsuitable soil or when they are attacked by a root fungus.

Where the growth of the fungus is slow and where infection has occurred on an older part a cankered appearance may be produced, representing an attempt on the part of the plant to heal a wound against the action of the fungus which tends to keep it open.

* Note more especially species of the genus *Eutypa*, of which *E. caulivora*, Massee, is a wound parasite on Para Rubber.

The time taken for a branch to die back varies necessarily according to its size and also according to the health of the branch and the position of the point of infection. Where infection occurs high up on a branch the length of the terminal portion of the branch which first dies, owing to the stoppage of the transpiration current, is small; where infection occurs near the base of a branch the death of the portion above the point of infection involves the death of nearly the whole of the branch.

On plantations, infected areas are usually scattered. Most usually one or two plants are infected here and there on an attacked area. It seldom happens that many adjacent plants are affected simultaneously.

The disease is more commonly met with, and the extent of the damage is greater in those areas in which the plants are unhealthy. On the whole it is more commonly met with on older rubber; it is common to find in areas consisting of old trees that the trees have a scanty foliage and are possessed of slow growth, whether this be due to a poor access of light, to excessive tapping, to the impoverished condition of the soil or to a combination of these influences. The relation of the disease to the health of the plant will be more fully discussed in a later chapter. For the present it may be said that the frequency of occurrence of the disease and the extent of the damage caused thereby are closely related to the general condition of health of the plants.

THE FUNGUS IN THE TISSUES.

The hyphae of the fungus can be readily distinguished microscopically in the tissues by their dark-brown colour, good staining effects are obtained in the wood by Delafield's haematoxylin and safranin and in the outer tissues by Delafield's haematoxylin alone.

In the wood the mode of growth of the hyphae varies according as to whether they are in the tracheids or medullary rays. In the tracheids they pursue a longitudinal course and are sparingly branched; in the medullary rays they branch repeatedly and give rise to a more abundant growth. The discolouration of the wood is due to the presence of the dark coloured mycelium.

Branches from the hyphae pass from one tracheid to another and from the tracheid to the cells of a medullary ray. The passage is effected through a pit. At the point of passage the hypha becomes constricted to about one-half of its breadth. On either side of the constriction there is a swelling of the hypha. Having reached the medullary ray the hypha branches freely and passes readily from one

ray cell to another. The hyphae are met with more frequently in the storage cells than in the conducting cells of the medullary ray.

The difference in growth of the hyphae in the tracheids and in the medullary rays appears, therefore, to bear a close relationship to the food content of these two tissues.

The solution of the starch in the medullary rays is readily effected.

There is no evidence of a solution of the walls of the tracheids or of the medullary ray cells except in so far as it concerns the passage of the hyphae from one cell to another.

The cells of the cambium are exhausted of their contents and the tissue is converted into a black mass produced by the growth of the hyphae.

The cells of the phloem and cortex are likewise invaded by hyphae which pass readily from cell to cell and can be commonly found in the intercellular spaces as well as within the cells. In the later stages of the disease the zone previously occupied by the cambium, phloem and inner layers of the cortex appears as an intensely black mass which becomes evident when the outer layers are removed.

Repeated examination has shown that the hyphae of the fungus may extend in the wood for a distance of four or five inches below the point where the wood can be seen by the naked eye to be dead; in other words, the wood is infected for another four or five inches below the dead part.

The growth of the hyphae in the wood was found to be always in advance of that in the outer tissues.

The death of the outer tissues is followed by the appearance of the fruits of the fungus. These take the form of black points arising singly or in a state of aggregation; they are just visible to the naked eye. They are globose, have a minute ostiole and measure on the average 180-200 microns in diameter. On the younger parts they are scattered, while on the older parts they are aggregated together with a stroma which is either smooth or covered with dark coloured hyphae. In these pycnidia are borne the spores which when young are hyaline and continuous and when old are dark-brown and once septate. The spores are abstricted at the ends of sterigmata which are mixed with filiform paraphyses; the paraphyses are hyaline and measure 100-130 microns in length. The average spore measurement is 24-27 × 13-15 microns. Biseptate spores sometimes occur; they are usually above the average measurement of the uniseptate spore. When an infected branch is kept moist the spores are frequently exuded in large numbers in tendril-like masses. When the spores

are thus ejected in a young condition the tendrils are white; they, however, soon darken and become finally of a sooty black colour.

The characters possessed by the pycnidia of the fungus on Para rubber differ in no respect from those of the fungus on cacao. In a preliminary note on the fungus* I pointed out that after making repeated comparisons between the *Diplodia* on cacao in West Africa and that on Para rubber in this country I was unable to detect any difference in the fructifications or mycelium of the two fungi, and I was, therefore, led to conclude that they were identical with each other. The characters of the spores and paraphyses and their measurements were quite similar, and the same variations in the state of aggregation of the pycnidia and in the nature of the stroma occurred in the specimens which were compared with each other. Further, the fungus on cacao had been previously examined at Kew and identified as *Diplodia cacaoicola*.

THE LIFE-HISTORY OF THE FUNGUS.

A series of experiments were conducted for the purpose of ascertaining the mode of germination of the *Diplodia* spores and the length of time taken for germination to commence. In these experiments hanging-drops were made use of, and the media employed were sterilised distilled water, sterilised tap water, a 1% solution of cane sugar in water and the latex of *Hevea brasiliensis*. Two dozen experiments were performed altogether, six in each one of the above media.

The earliest record of the commencement of germination was in eight hours after the spore had been transferred to the drop; this took place in tap water; on the whole, however, the nature of the medium did not appear to influence the initial phases of germination. Germination in all of the media was found to occur freely within twelve hours.

On germination each spore emitted, as a rule, one hypha; two hyphae, one from each cell, occurred less frequently. The hypha grew and branched. In tap water and distilled water the growth was soon checked, but in latex and cane sugar solution an accumulation of mycelium occurred. The mycelium was composed of hyphae which were hyaline at first and which assumed a brown colour as they became older.

It was found that germination could occur before the spore became septate.

* Agricultural Bulletin, S. S. and F. M. S., vol. IX, no. 12, p. 475.

Various media have been tried for the preparation of cultures; these are an extract of the juice of the cacao pod diluted ten times, a 20% solution of the extract of shoots of Para rubber, a medium composed of cane sugar 1%, calcium nitrate, magnesium sulphate, sodium phosphate and potassium chloride, each .25%, and a 10% solution of the juice of the sugar cane. A growth of the mycelium of the fungus was found to occur in all of these media, but the growth obtained in the solution of the juice of the sugar cane was more active than in any of the others, and this was selected as the medium for the production of cultures for inoculation experiments. This medium was used diluted one-half with 1½% agar-agar or diluted one-half with water. In the liquid cultures the growth of the mycelium was more rapid. By a dilution method it was found possible to obtain cultures in which the growth emanated from a single point, which when examined was found to correspond in position with a single *Diplodia* spore.

The mycelium obtained was at first white; it became darker in colour and was eventually almost black. It was found to be composed of dark-brown hyphae, 5 microns in width, repeatedly branched and septate at moderately frequent intervals. The mycelium became aggregated in spots which increased in size and developed into the pycnidia of *Diplodia*. The following note on one of the liquid cultures will illustrate the time changes which took place:—

Thurs. Aug. 25. Culture prepared.
„ 27. White mycelium observable.
„ 28. Mycelium darkening.
„ 30. Mycelium aggregating in spots.
Sept. 2. Spots evident as pycnidia.
„ 5. Pycnidia producing spores.

The spores from the pycnidia were used for inoculation, the pycnidia being removed from the culture and crushed in a drop of sterilised distilled water.

In the first series of inoculation experiments attempts were made to produce infection at an unbroken surface. Seedling plants, five months old, were used in these experiments. The spores were transferred to the plants by two methods,—in the one they were mixed with sterilised distilled water in an atomiser and sprayed over the entire surface of the plants, and in the other they were transferred in a drop of water on to definite spots on the leaves and young shoots by means of a sterilised pipette, each spot being kept marked by means of a gummed label. Four plants were sprayed with spores on September 2nd and two plants were kept as controls. On September 9th

the experiment was repeated. On September 2nd four plants were treated with spores by direct apposition on definite spots, and two plants were kept as controls. On September 9th this experiment was repeated. In each plant the leaves as well as the stems were treated with the spores.

At the end of two months the plants were found to have developed no symptoms of disease, and, further, a microscopic examination failed to show the presence of any fungus mycelium in the tissues. It was, therefore, concluded that infection could not occur at the unbroken surface.

In the second series of experiments attempts were made to inoculate at a wounded surface. Seedlings of five months and of one year were employed. The wound was made by removing a flap of tissue, at a point approximately three inches from the apex, the cut being from above downwards and deep enough to expose the wood. The spores were transferred to the wood and the spot was moistened with sterilised distilled water; the wound was then bound up with water-tight tape.

On September 3rd four plants were thus inoculated; they were five months old. Two plants of the same age were similarly wounded but not inoculated; they served as controls. On September 8th Nos. 1 and 2 showed symptoms of disease in the form of a withering of the top part of the shoot; Nos. 3 and 4 showed this on September 9th. The death of the top shoot occurred on September 9th in 1 and 2 and on September 10th in 3 and 4. On the dead part pycnidia of the *Diplodia* were found on September 14th.

The death of the plant was continued downwards and had reached the base of the plant in eighteen days in No. 2, twenty days in No. 1 and twenty two days in No. 3.

In No. 4 the dying back proceeded downwards for a distance of two inches below the point of infection and then ceased. The plant produced a vigorously growing shoot below this point and the further action of the fungus was checked. The controls showed no signs of disease.

A similar experiment was commenced on September 6th. Six plants of five months of age were employed. Nos. 1, 2, 5 and 6 yielded in all respects similar results to those obtained in Nos. 1, 2 and 3 above. The time taken for the death of the terminal portion of the shoot was from six to eight days from the time of infection, and the death of the plants was recorded in from sixteen to twenty-two days after infection. Nos. 3 and 4 produced initial symptoms of disease similar to those which occurred in Nos. 1, 2, 5 and 6, but after a time the dying back was checked and

growth was continued by a subsidiary shoot which arose from below the dead part.

Of the ten plants infected in these two experiments seven were killed outright and three developed initial symptoms of disease and eventually recovered.

On September 8th six plants, one year old, were infected. The distance of the point of infection from the apex of the shoot was eight inches. The death of the terminal portion of the shoot was recorded in from fourteen to twenty days after infection. The dying back extended downwards and in Nos. 1, 3 and 6 reached the base of the plants. The time taken for the plants to die was in No. 1 fifty-four days, in No. 3 sixty days and in No. 6 sixty five days. No. 2 developed no symptoms of disease.* No. 4 died back to a distance of three inches below the point of infection and then ceased to do so, growth being continued by a shoot developed below this point. No. 5 behaved similarly to No. 4, the dying back extending further, however, and reaching a point seven inches below the point of infection.

The results of these experiments show that the *Diplodia* is capable artificially of infecting the plant at a wound with the production of symptoms of disease similar in all respects to those observed on plants which are concluded to be infected naturally. The complete death of the plant does not always occur; the dying back may in some cases be checked and growth may be continued by an accessory shoot.

In the wound inoculations performed above, the wound was sufficiently deep and large to expose an area of wood of at least 1 cm. long and .5 cm. broad. Another series of experiments was conducted for the purpose of ascertaining the size and depth of the wound required for infection.

On October 21st four plants were inoculated at small pricks on the stem. The spores were placed in the drop of latex which exuded on pricking the stem with a needle. Three pricks were made on each plant at a distance of about four inches from the apex. The plants were in good health and were approximately five months old. An examination at the end of six weeks showed that the plant had not developed any symptoms of disease and the experiment was then abandoned.

On October 22nd four plants were inoculated at small wounds produced by removing a small area ($\frac{1}{2}$ cm. long and $\frac{1}{4}$ cm. broad) of the outermost tissues of the stem by a razor.

* This was probably due to the absence of spores in the drop of liquid which was placed on the wound,

The plants were aproximately five months old and were in good health. An examination at the end of six weeks showed no signs of disease in any of the plants.

From these experiments it was concluded that infection does not occur with any degree of facility, if at all, at small wounds which do not expose the wood of the plant.

Attempts were made to inoculate at the tapping surface, and in these only well tapped surfaces were employed, since it has been shown before that infection can take place where the wood is exposed. A well tapped surface is considered in this case as one in which the cut does not extend sufficiently deeply to cause the death of the cambium and of the secondary phloem adjacent to it, and the consequent exposure of the wood. Tapping surfaces one day old, two days old and three days old were employed, and the ages of the plants were seven and nine years. The spores were placed in a drop of water on the surface and were kept moist for three days. On each plant spores were placed on the one-day-old tapped area, the two-day and the three-day. Six plants were used, thus making eighteen experiments altogether. In no case was it found that infection occurred on any of these areas after examination at the end of six weeks, and an examination of the tissues underlying the spots inoculated showed no fungus mycelium. It was, therefore, concluded that infection does not occur on a well tapped surface.

To summarise the conclusions based on the results which have attended these inoculation experiments—the Diplodia spore is capable of infecting the plant at a wounded surface and of producing symptoms of disease which are similar to those which follow on infection in a state of nature; the time taken for symptoms of disease to become apparent after infection has occurred varies according to the distance of the point of infection from the apex of the shoot, and the time taken for the plant to die varies according to its age, being in the five months seedlings approximately three weeks and in the one-year-old plants eight or nine weeks; the death of the plant does not necessarily result from infection, the dying back in some cases being checked and the growth of the plant being continued by an accessory shoot developed from below; infection does not occur at small, simple wounds which do not lay bare the wood of the plant nor on the well tapped surface; the *Diplodia* reproduces itself by producing pycnidia on the dead parts.

This concludes the investigations which have been made on the parasitism of the *Diplodia*. It remains to show whether there are any other spore-forms, and how these

are related to the *Diplodia* and to the "die-back" disease which is caused by it.

On diseased plants no other spore-forms have occurred which have suggested themselves as being connected with the *Diplodia* except a *Fusarium*-form which was observed in some abundance on samples of cacao attacked by *Diplodia* in West Africa. The *Fusarium* was confined to the portions of the bark containing the stromata of *Diplodia*, each stroma being, as it were, capped by a white covering of the *Fusarium*. There was no time to investigate the development of the *Fusarium* in artificial cultures. *Fusarium*-forms have been mentioned by more than one author as occurring on cacao; it is more likely, however, that they are connected with the *Nectria spp.* on the cacao plant. I have not found a *Fusarium*-form to accompany the *Diplodia* in this country.

Specimens consisting of diseased branches of cacao were received at Kew from West Africa in the early part of 1910; on these the *Diplodia* was fruiting abundantly. In order to watch the development of the fungus some of these specimens were placed in a sealed jar and kept moist with a piece of cotton wool which was soaked in distilled water so as to ensure their drying slowly. They were examined at the end of four months, at the end of five months and at the end of six months, the position of the stromata of the *Diplodia* having been previously carefully marked. At the end of six months it was found that the stromata were giving rise to an ascigerous fungus. The stromata altered slightly, becoming slightly lighter in colour, but still retaining their covering of hyphae. The perithecia were produced in the stromata, measured less than those of the *Diplodia* (150 microns) and bore asci which were cylindric-clavate, sessile, 90-100 × 12 microns and were mixed with filiform paraphyses 100-130 microns long; the spores were obliquely monostichous to distichous, oblong, fuliginous, three-septate, slightly constricted at the septa and measured 19-20 × 6-7 microns.

The fact that this ascigerous fungus was produced on the same stromata as had previously given rise to pycnidia of *Diplodia*, coupled with the fact that on some stromata it was possible to find old pycnidia which had not yet lost all of their *Diplodia* spores, appeared to leave little or no room for doubt that it was the ascigerous condition of the *Diplodia*.

From the characters of the stroma, perithecia, asci and spores I have classified this form in the genus *Thyridaria*, Saccardo, and have designated it a new species *Thyridaria tarda*. The genus *Thyridaria* was founded by Saccardo

in 1875. Pycnidial (*Diplodia*) and micropycnidial (*Coniothyrium*) forms are known to occur in species of the genus.

For the purpose of ascertaining the product of development of the ascospore and its relation to the *Diplodia*, artificial cultures were made from the ascospore.

The capacity for germination was first tested by mounting the spores in hanging-drops. Germination was found to take place readily in one and a half days, the estimated age of the spores being four weeks. Two or three cells most usually emitted one hypha each. In a 1% solution of cane sugar in the drop the hyphae were found to grow freely and to become dark-brown in colour as they got older.

By removing a stroma and crushing it in a drop of sterilised water, the ascospores were obtained, and artificial cultures were made in 5% cane juice and 1½% agar-agar. By a dilution method it was found possible to obtain cultures of mycelium which emanated from a single point, which point was found to correspond with the position of an ascospore. The mycelium was composed of hyphae which were at first hyaline and soon assumed a dark-brown colour. They were repeatedly branched, were septate at fairly short intervals, measured on the average 5-6 microns in width and were similar to the hyphae of *Diplodia* when grown in the same medium.

Some of the hyphae were removed from the culture and were transferred to wounds on Para rubber plants of six months of age, the wounds being kept moist with distilled water for three days. Six plants were employed, of which five were thus inoculated and one was used as a control. One wound was made on each plant and was approximately three inches below the apex. At the end of eight days after inoculation two plants showed symptoms of disease, and after eleven days from the time of inoculation all of the plants were diseased. The symptoms were the death of the apex of the shoot above the wound and the subsequent death of the shoot down to its base; the death of the whole shoot was recorded in all five cases.

On the dead parts of the plants were produced black pycnidia which were just visible to the naked eye, arising singly and becoming erumpent through the epidermis. The pycnidia were found to contain dark-brown, one-septate spores. The nature of the pycnidia and the characters and measurement of the spores were in all respects similar to those of the *Diplodia* which were produced by inoculation with the *Diplodia* spores from Para rubber.

The results of the experiments showed that the ascospore from the cacao plant was capable of infecting the Para rubber plant with production of symptoms of disease

and of pycnidia identical with those which were obtained when inoculation was produced by using spores of *Diplodia* from Para rubber. These results not only showed that the ascospore could reproduce the *Diplodia* form on the plant, but further served to establish the identity which had been supposed to exist between the *Diplodia* on cacao and that on Para rubber.

The further progress of the mycelium derived from the ascospore was carefully studied in the cultures on cane juice-agar; and it was found that, although this resembled in many respects the mycelium which was reproduced from the *Diplodia* spore in the same medium, there was no production of pycnidia of *Diplodia* at the end of eight weeks. On the other hand it has been shown that if the mycelium be passed through the living plant the production of pycnidia of *Diplodia* occurs rapidly on the dead parts of the plant. Whether this may be due to the nature of the artificial nutrient medium employed or to the necessity for infecting the living plant before the ascospore can give rise to the *Diplodia* condition it is not possible to say, owing to the fact that the experiments are too few to enable me to draw any definite conclusions.

The transition of the *Diplodia* to the ascigerous condition has never been found to occur in artificial media, although some of the cultures are more than twelve months old. It has only been found on a natural medium, and here it has been obtained by allowing the *Diplodia* to have full play and the medium to gradually desiccate.

A search on old dead shoots of Para rubber has revealed the presence of the ascospores of the fungus in small quantity, the material having been obtained from a tree which had been infected with *Diplodia* and was some time dead.

The abundance of material and more ample time for observation has enabled me recently to make a more careful study than was hitherto possible of the changes which take place in the stromata subsequent to the production of the *Diplodia* stage; and, although there has not been sufficient time for the ascigerous stage to develop, yet the investigations have revealed the presence of a small pycnidial form which, by virtue of its position and by the fact that it has occurred freely on the specimens investigated, I have concluded to be connected with the *Diplodia* condition.

The material for these observations consisted of three specimens of the trunk of a three year old tree of Para rubber which had been infected with the fungus. They were treated in a similar manner to the specimens of cacao previously employed, *i.e.* were moistened and allowed to

desiccate slowly under a bell-jar. The observations were conducted by marking the positions of the stromata and examining microscopically by hand sections some of the stromata at intervals of three weeks. The pycnidia of *Diplodia* gradually ejected their spores and remained as empty sacs containing, perhaps, a few *Diplodia* spores which had not been ejected. After nine weeks, the specimens having been dead for approximately three months, the stromata revealed the occurrence of a pycnidial form with minute spores. The pycnidia were present on the stromata along with the old *Diplodia* pycnidia, thus exhibiting a transitional stage in the passage from one form to the other. They were black and submembranaceous, ovate, with a distinct ostiole; they were aggregated on a stroma and were rough above with dark-brown hyphae; they measured 200-250 × 180-200 microns and the ostile was 30 microns wide. The pycnidia contained abundant sterigmata, 22-24 × 2-3 microns, which bore at their ends elliptic-oblong hyaline spores measuring 3-4 × 1½-2 microns. From the characters of the pycnidia and spores this form has been identified as belonging to the form-genus *Cytospora*.

The investigations on the fungus are being continued with a view to demonstrating the transition from the *Cytospora* to the ascigerous form.

From these investigations on Para rubber and on cacao it has been concluded that the life-history is made up of three forms,—one a *Diplodia* which appears to be the form destined for rapid reproduction and the parasitic form, another a *Cytospora* which develops on the plant some time after it has died, and the third a *Thyridaria* which appears later and which can infect the living plant with production of the *Diplodia*. The fact that the *Cytospora* was not demonstrated on the cacao material is considered to be due to lack of opportunity for repeated and careful examination.

RELATION OF PARASITE TO HOST.

From a study of its mode of life the fungus is regarded as being normally wont to pass its existence as a saprophyte. It must be included in the class of fungi designated by De Bary as "facultative parasites" and by Tubeuf as "hemisaprophytes"—a class including those fungi which under certain conditions are capable of living as parasites. It remains, then, to analyse the conditions which are necessary for parasitism. The fungus has been shown to be capable of penetrating into the host only at a wounded surface, *i.e.* it must commence its existence on dead tissue.

It is necessary to distinguish between a simple wound which does not expose the central woody core of the plant and a wound which lays bare such a complex mass of tissue as the wood. In the former case the injury involves the death of the cells exposed and the cells under these develop a protective covering of cork which may or may not be retained by the plant. In the latter case the cells bordering on the wound are similarly affected; the exposed cells of the wood are, however, incapable of growth; they die, and the healing of the wound is produced by the development of a tissue, the product of the cambium, which eventually grows over and covers the exposed wood. Not only then is the damage which follows on the exposure of the wood more serious, but it is also more permanent. These considerations furnish an explanation to the results which attended certain of the experiments described above. In these it was shown that infection did not occur at the wounds produced either by removing one or two of the outermost layers of the stem, or by pricking the stem with a needle and germinating the spores in the latex, or by germinating the spores on a well tapped surface. In these cases the dead tissue is rapidly shut off from the living tissue underneath and this does not allow of the accumulation of sufficient mycelium on the part of the fungus for it to spread to the healthy tissues adjacent to the wound. The exposure of the wood, on the other hand, results in the production of a dead tissue on which germination readily occurs; a mass of mycelium is accumulated and the fungus spreads from the dead tissue to the living tissue adjacent to it. It has also been shown that the extent of diseased wood is always greater than that of the outer tissues.

The saprophytic nature of the fungus has been emphasised above. Even when it is exerting its influence as a parasite on the living tissues its inherent saprophytic nature becomes prevalent, when, having killed off the portion of a branch above the point of infection by blocking the ascent of sap to that part, it spreads to the dead part far more rapidly than it can proceed downwards from the point of infection to the living tissues below.

Similar results attended certain other wound infection experiments. In these, infection was obtained at a wound made by removing the bark; the area of wood which was occupied by the growth of the fungus was measured at the end of three weeks; the hyphae of the fungus were found to have extended for a distance of three inches to four inches above the point of infection; this was ascertained by removing and examining microscopically small portions of the wood. The wood above this point was then laid bare

by tearing off the bark, the bark being removed for a distance of three and a half inches in length and one inch in breadth. The area of wood so exposed became discoloured by the growth of the hyphae in from four to five days. In other words, whereas it had taken the hyphae three weeks to spread from the point of infection for a distance of from three and a half to four inches up the wood when the wood was healthy, it only required four to five days for them to accumulate an equal growth when the wood was dead. Three plants were treated in this manner; they were one and a half years old, and the inoculations were made on the trunk five feet from the ground.

These experimental observations are here given because they are considered to have a distinct bearing on the relation which exists between the fungus and the condition of health of the plant attacked,—a relation which I consider to be determined on the one hand by the prevalent saprophytic nature of the fungus as compared with its capacity for exerting a parasitic influence, and on the other by the capacity on the part of the plant for resisting the growth of the fungus.

Such trees as are poor in foliage, as are capable only of slow growth and as are easily affected by adverse climatic or soil conditions lend themselves more readily to attacks from the disease, in that not only is the fungus more easily able to initiate the disease, but that it is capable of causing more extensive damage by virtue of the low degree of resistance offered by the plant. Nor is this condition of affairs infrequently met with among fungi,—witness such a case as the disease of lettuce in temperate regions caused by *Botrytis cinerea*, a fungus which occurs abundantly as a saprophyte and which can only cause a disease of lettuce when that plant is grown under unfavourable conditions, as in a hot-house devoid of proper ventilation and kept excessively damp. Other instances are furnished by species of *Nectria, Cucurbitaria* and *Phoma,* and on ripe fruits by species of *Mucor* and *Penicillium.*

To sum up these observations it may be said that the further the plant is from its full condition of vital activity the more liable is it to attacks from the disease caused by the *Diplodia* condition of the fungus.

THE NOMENCLATURE OF THE *DIPLODIA* CONDITION OF THE FUNGUS.

In the account which is given of the history of the fungus at the beginning of this work it will seen that the fungus has been classified in four different genera—*Dip-*

lodia, *Botryodiplodia*, *Lasiodiplodia* and *Macrophoma*—and that Griffon and Maublanc have concluded, after an examination of the original specimens of *Botryodiplodia Theobromæ* and *Macrophoma vestita*, that these two fungi are identical and that they are the same as *Diplodia cacaoicola* and *Lasiodiplodia nigra*.

In the genus *Macrophoma* the spores are hyaline and continuous, not dark-brown and once septate as they are in the other three genera. The spores of the *Diplodia* are hyaline and continuous when young, and it must, therefore, be concluded that the misnaming in this case was due to the examination of unripe specimens.

In the other three genera the differences on which the generic characters are based are the state of aggregation of the pycnidia and the nature of the stroma. In *Diplodia*, founded as a genus by Fries in 1849, the pycnidia are scattered; in *Botryodiplodia*, Saccardo, 1880, the pycnidia are aggregated on a basal stroma; in *Lasiodiplodia*, Ellis and Everhart, 1896, the pycnidia are included in a stroma which is invested with a black mycelium above. In the species under consideration the pycnidia as a rule are scattered on the young parts of the plant, while on the older parts they are aggregated and may then be provided with a basal stroma, which is more or less smooth, or may be included in a stroma which is invested above with a black mycelium. In short, according to the state of aggregation of the pycnidia and the nature of the stroma this fungus may be classified in any of the above genera. Yet even if one were to be inclined, on such scanty evidence as this, to suggest the abolition of two of the genera *Diplodia*, *Botryodiplodia* and *Lasiodiplodia*, the oldest generic name *Diplodia* must stand. The parasitic condition of the fungus must, therefore, of necessity be placed in the form-genus *Diplodia*.

The variation in the state of aggregation of the pycnidia and in the nature of the stroma has in all probability led to this multiplication of names for the fungus,—an occurrence which, apart from having a certain academic significance, has tended to prevent the recognition of the wide distribution of the species.

The paraphyses are always present along with the spore-bearing hyphæ. In the original description of the genera *Diplodia*, *Botryodiplodia* and *Lasiodiplodia*, paraphyses are only mentioned in the last named genus; Hennings, however, mentions the occurrence of paraphyses in his description of *Diplodia cacaicola*.

The size of the spores appears to vary somewhat according to the measurements recorded by different authors.

For *Botryodiplodia Theobromae* the measurement given is 25-35 × 12-15 microns, for *Macrophoma vestita* 30 × 15, for *Diplodia cacaoicola* 22-28 × 12-14, for *Lasiodiplodia nigra* 28-32 × 18-21, for *Diplodia rapax* 32-35 × 15-16, and for *Botryodiplodia elasticae* 25-30 × 14-15. Howard mentions that the spores of the *Diplodia* on cacao in the West Indies were very constant in size and measured 20 × 10 microns. I have had an opportunity of measuring some of the spores of the original *Diplodia* from Grenada, West Indies, and have found them to average 23 × 13 microns; the measurements were made by means of a micrometer screw. The average spore measurement of the fungus in this country is 24-27 × 13-15 microns.

SOURCES OF INFECTION.

There are two sources by which infection is derived, the source from which the spores are produced and that by which the fungus effects an entrance into the host.

With regard to the former it has been shown that the fungus exists in abundance on the dead remains of plants of many kinds and that the spore is capable of germinating in artificial media and of producing pycnidia in eleven days. Dead branches which are allowed to lie about furnish a source of infection, as likewise do diseased branches which are allowed to remain on the tree.

With regard to the latter source, it has been shown that the fungus must commence its existence on dead tissue. Such dead tissue is furnished by wounds arising from various causes and by dead parts which are allowed to remain on the plant. It will be necessary to enumerate the wounds which occur most commonly on Para Rubber in this country.

In the planting out of stumps, the cut end of the stump presents a wounded surface. When growth commences again a shoot is produced below this cut surface and becomes the main axis of the plant. The portion of the stem above the point of origin of the shoot is cut off from the transpiration current and dies; this dead portion affords a means of entrance for the fungus.

The practice known as "topping," which consists in the removal of the upper portion of the main axis of the shoot, exposes a wounded surface, and eventually, when a number of lateral branches have arisen below the topped part, owing to the concentration of the weight at one point a splitting frequently occurs down the trunk and exposes the wood. "Topping" is a severe method of pruning for which there appears to be little or no excuse.

Pruning, either thumb-nail or the removal of small or large branches, exposes a wounded surface.

Corticium javanicum, the "pink fungus," frequently produces a disintegration of the bark and exposes the wood beneath. Attacks by *Diplodia* frequently follow on attacks by the "pink fungus."

As the trees grow older the lower branches die off owing to excessive shading. These dead branches remain attached and as such provide the fungus with a means of entrance.

Other sources, such as wounds produced by animals and by exposure of the wood in tapping, also occur.

In addition to wounds may be mentioned the action of root fungi which weaken the plant and render it more susceptible to the disease. For this reason *Diplodia* frequently follows on an attack by *Fomes semitostus* or by *Hymenochaete noxia*. The occurrence of fructifications of the *Diplodia* on a tree, then, does not necessarily indicate that the disease was initiated by this fungus. In fact, the fungus comes on readily on plants which are dead or dying, and possesses such an active capacity for growth that it tends to mask the real cause of the trouble. In view of this it is extremely likely that by no means all of the effects which are attributed to this fungus have been initiated by it.

WORK OF PREVIOUS AUTHORS.

Howard first investigated the parasitism of the fungus in the West Indies in 1901 and showed that it was only capable of entering the stem through a wound. He considered that it was not likely to cause serious damage to well-tended, vigorous trees, and hence he regarded the establishment of a healthy condition of the plant as being of primary importance in connexion with the disease. He also showed that the fungus could be transferred from cacao to sugar cane.

Then followed work by Charles on the fungus on mango from San Domingo, by Stockdale on a stem and root disease of cacao in the West Indies attributed to a species of *Lasiodiplodia* which is now considered to be identical with the *Diplodia* dealt with in this work, by Appel and Laubert on the fungus on cacao and papaw in Samoa and by Griffon and Maublanc on the various synonyms of the fungus.

In 1910 Ridley published the results of certain infection experiments on the fungus under the name of *Diplodia rapax*. He found that although the fungus was unable to damage the terminal bud directly, it could do so if the bud were previously injured in any way; that it could produce

direct infection on very young leaves; that if the plant were unhealthy it was killed outright in a few days, but in the healthy seedling the progress of the fungus was checked; that slight wounds on woody parts did not provide a means of entrance for the fungus; and, finally, that the fungus is capable of initiating disease without the aid of any other fungus. It is important to notice that, although Ridley's experiments were few in number, they go to show that there is in some cases a definite recovery on the part of the diseased plant. The results which attended his experiments differ from mine in the time taken for the plant to die after inoculation and in my inability to produce artificial infection on the young uninjured leaves.

In 1910 Petch described a disease of *Hevea brasiliensis* in Ceylon. He attributed the early stages of the disease to *Gleosporium alborubrum* and the later stages to *Botryodiplodia elasticae,* a species which, as has been mentioned before, he regards as being identical with the *Diplodia* on cacao. I have not recorded the presence of *Gleosporium alborubrum* in this country, and at the same time I have been able to shew that the *Diplodia* can initiate the disease independently of another fungus. Any fungus, however, which effects the death of the tissues of the plant would provide a means of entrance for the *Diplodia*. Petch also reports that in Ceylon the fungus causes a root disease of tea and a stem disease of *Albizzia moluccana* and can infect at wounds on dadap (*Erythrina*).

In addition to the above mentioned hosts the fungus has been reported on *Castilloa elastica* in the West Indies and is considered to cause a root disease of coconut in Trinidad.

METHODS OF TREATMENT.

The methods of treatment follow naturally on a knowledge of the fungus, its mode of life and its effect on the host.

The occurrence of the fungus on dead wood of Para rubber neecssitates the sanitation of the plantations, both in places where the disease is known to occur and in places where it has not yet been met with. Dead branches should not be left lying about, but should be gathered into small heaps and burnt on the spot. Dead parts of other plants which may be growing along with the rubber, such as coconut, coffee, etc., should be treated in a similar manner. Such a method of sanitation reduces the spore production of the fungus,

The fungus causes greater damage to unhealthy trees than to healthy ones. Attempts should, therefore, be made to ensure a vigorous growth on the part of the plant by practising good cultivation. A good access of light should be obtained by avoiding close planting. In fields of six years and more a planting distance of twenty-five by twenty feet appears to be sufficiently close; this gives eighty-seven trees to an acre. Excessive tapping should be avoided. A good subsoil drainage should be obtained. The application of artificial fertilisers might well be practised among older rubber. In this connexion the application of lime may be mentioned. One would expect many of the soils in this country to be deficient in the content of lime, and the analysis of soils which have hitherto been made by the Chemist to this Department have shown a deficiency in lime, some soils containing only one-tenth of the minimum amount of lime which a fertile soil should contain, *i.e.* 1%.

As a preventive measure all wounds should be tarred, and by a wound is meant any lesion which exposes the central woody core of the plant. Special care should be taken to tar the cut ends of stumps, and to remove such lower branches as may die off owing to excessive shading and to tar the surface of the trunk which is thus exposed. Coal tar should be used; it possesses two distinct advantages above Stockholm tar, in that it is more permanent and offers better protection against fungi. The tar should be warmed slightly before using and should be applied by means of a brush in the form of a thin layer. It should not be allowed to run down the sides of the branch or trunk. The cut surface should be allowed to dry slightly before it is tarred.

Diseased branches should be removed. The indication of the extent of disease is furnished by the wood. In removing a diseased branch it is necessary to cut back until a point is reached where the wood is not discoloured and then to remove another six inches of the branch. A sloping surface should always be left after removal. In removing a large branch it will be advantageous to make more than one cut. First, a cut should be made eight or ten inches from the trunk from below upwards and should go half-way through the branch; then, a second cut should be made two inches further from the trunk from above downwards and should go through the branch. Finally, the stub should be sawn off flush with the trunk. Stubs must not be left, since they never heal over and invariably die back to the trunk. All large branches must be removed by means of a saw.

Deep tapping and consequent exposure of the wood should always be avoided.

SUMMARY.

The results of the investigations on the fungus may be briefly summarised as follows:—

(1). The fungus is widely distributed through the cacao and rubber producing countries of the tropics. In addition to cacao and Para rubber it has been recorded on *Albizzia moluccana*, *Erythrina umbrosa* and tea in Ceylon, on sugar cane, *Castilloa elastica* and coconut in the West Indies, on mango in San Domingo and on papaw (*Carica papaya*) in Samoa; it also develops readily on dying or dead parts of many plants.

(2). The fungus is a wound parasite, and the disease is caused by the *Diplodia* condition. The *Diplodia* disappears on the dead parts some time after they are dead and a micropycnidial form takes its place. Finally, at the end of about eight months after the death of the plant, an ascigerous form makes its appearance. The ascigerous form is a *Thyridaria* and bears the name of the fungus, *Thyridaria tarda*, of which the two pycnidial forms, *Diplodia* and *Cytospora*, are stages. The ascospore can infect the living plant with production of disease and with formation of the *Diplodia* condition of the fungus.

(3). The fungus must commence its existence on dead tissue, and having accumulated a mass of mycelium it spreads to healthy parts with consequent production of disease. Having become inoculated the plant is sometimes capable of recovery; the dying back is checked and growth is continued by an actively growing shoot which arises from below the dead part. The damage done by the fungus is closely related to the condition of health of the plant, and this is more evident in the case of older plants.

The following is given as a diagnosis of the fungus:—

Peritheciis monostichis, nonnullis minoribus saepe superimpositis, stromate atro erumpente villosulo immersis, ostiolo minuto donatis; ascis cylindraceo-clavatis, octosporis, sessilibus, 90-100 × 12 microns; paraphysibus (100-130 microns longis), copiosis filiformibus obvallatis; sporidiis oblique monostichis, oblongatis, fuligineis, triseptatis, leniter constrictis, 19-20 × 6-7 microns.

Diplodia et *Cytospora* adsunt.

Diplodia—Pycnidiis submembranaceis, globosis, atris, ostiolo minuto donatis, 180-200 microns diam.; sporis ellipticis, utrinque obtusis, haud constrictis, fuligineis, 24-27 × 13-15 microns.

Cytospora—Pycnidiis submembranaceis, atris, ovatis, stromate atro ruguloso immersis, otsiolo prominulo (30 microns lato) donatis, 200-250 × 180-200 microns; sterig-

matibus copiosis, 22-24 × 3 microns; sporis ellipticis, hyalinis, 3-4 × 1.5-2 microns.

A list of terms synonymous with the *Diplodia* condition is appended:—

Botryodiplodia Theobromae, Pat.
Macrophoma vestita, Prill. and Del.
Diplodia cacaoicola, P. Henn.
Lasiodiplodia nigra, Appel and Laubert.
Lasiodiplodia Theobromae, Griff. and Maubl.
Botryodiplodia elasticae, Petch.
Diplodia rapax, Massee.

AUTHORS EXAMINED.

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Massee.—*Kew Bulletin*, No. I, 1910, p. 3.

Ridley.—Agricultural Bulletin of the Straits and Federated Malay States, Vol. IX, 1910, p. 290.

Petch.—Circulars and Agricultural Journal of the Royal Botanic Gardens Ceylon, Vol. IV, No. 23, 1910.

DESCRIPTION OF PLATES.

Plate I.

Fig. 1. A transverse section of a stroma of the *Diplodia*-form shewing three pycnidia and the covering hyphae on the surface of the stroma.

Fig. 2. A single, isolated pycnidium of the *Diplodia*-form shewing the spores and paraphyses.

(Fig. 1 × 75. Fig. 2 × 250.)

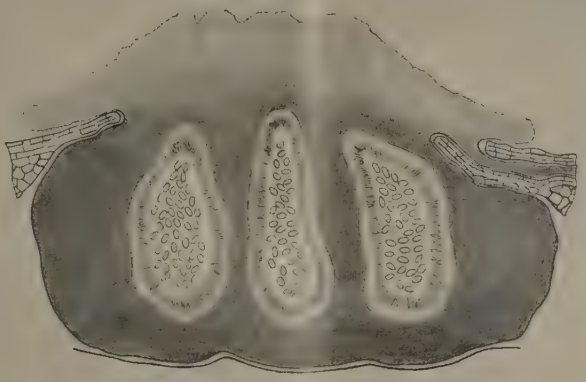
PLATE II.

FIG. 1. Spore-bearing hyphae and a paraphysis of the *Diplodia*-form.
FIG. 2. Three mature spores of the *Diplodia*-form.
FIG. 3. Two biseptate spores of the *Diplodia*-form.
FIG. 4. A transverse section of a stroma bearing perithecia of the ascigerous form—*Thyridaria tarda*.
FIG. 5. Asci and paraphyses.
FIG. 6. A single ascus with paraphysis.
FIG. 7. Mature ascospores.
(FIGS. 1, 2, 3 and 6 × 250; FIG. 4 × 60; FIG. 5 × 150; FIG. 7 × 500).

PLATE III.

FIG. 1. Early stages in germination of two *Diplodia*-spores.
FIG. 2. Old mycelium of the *Diplodia*-form from a culture.
FIG. 3. Hyphae of *Diplodia*-form in the cortex of *Hevea brasiliensis*.
FIG. 4. A germinating ascospore.
FIG. 5. Old mycelium derived by germination of an ascospore, taken from a culture.
FIG. 6. A single pycnidium of the *Cytospora*-form.
FIG. 7. Spores of the *Cytospora*-form.
(FIGS. 1, 2 and 5 × 250; FIGS. 3 and 6 × 200; FIG. 4 × 500; FIG. 7 × 600).

PLATE I.



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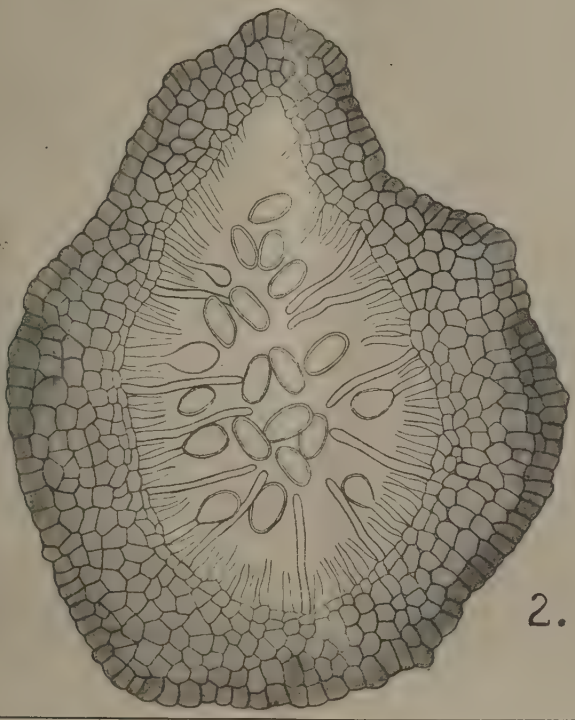

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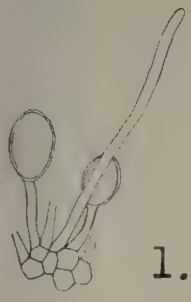

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PLATE III.

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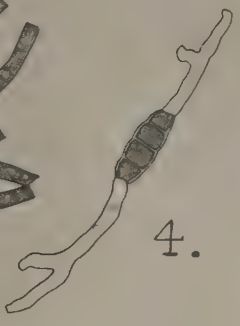

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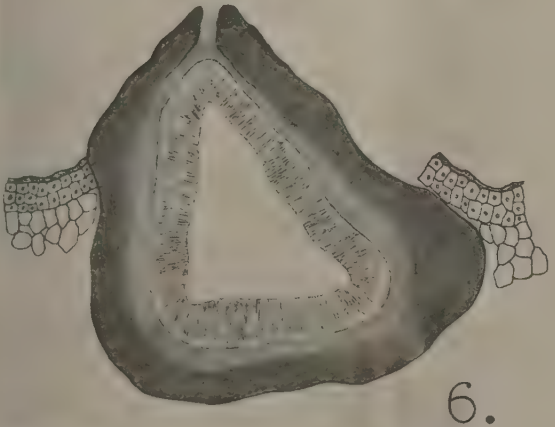

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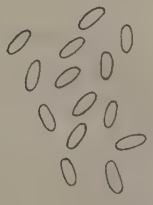

7.

The following Bulletins have been published by the Department of Agriculture.

1. Notes on *Termes Gestroi* and other species of Termites found on Rubber Estates in the Federated Malay States: by H. C. Pratt, Government Entomologist.

2. Root Diseases of *Hevea braziliensis*, the Para Rubber Tree: by W. J. Gallagher, Government Mycologist.

3. Observations on *Termes Gestroi* as affecting the Para Rubber Tree, and Methods to be employed against its Ravages: by H. C. Pratt, Government Entomologist.

4. A Lepidopterous Pest of Coconuts, *Brachartona catoxantha* Hamps, (Zygaenidae): by H. C. Pratt, Government Entomologist.

5. The Extermination of Rats in Rice-Fields: by W. J. Gallagher, Government Mycologist.

6. A Preliminary note on a Branch and Stem Disease of *Hevea Braziliensis*: by W. J. Gallagher, Government Mycologist.

7. Coffee robusta: by W. J. Gallagher.

8. The Cultivation and Care of the Para Rubber Tree (in Malay).

9. Die-Back Disease of Para Rubber and of Cacao: by Keith Bancroft, Mycologist.

10. A Lecture on the Para Rubber Tree: by W. J. Gallagher.

11. Coconut Cultivation: by L. C. Brown, Inspector of Coconut Plantations.

12. Padi Cultivation in Krian; by H. C. Pratt, Government Entomologist.